

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

CLAIMS

1. (Currently amended) A method for determining the rate of transcription of a transcriptional unit in a composition of cells, said method comprising:

lysing the cells and obtaining from the cells a preparation of nuclei comprising said transcriptional unit with nascent RNA strands attached thereto and placing same on ice to temporarily inhibit continued transcription and then placing said nuclei under conditions to permit transcription of the transcriptional unit in the presence of biotin-16-UTP, wherein said biotin-16-UTP includes a cleavable linker between the biotin and the UTP, to thereby provide a population of biotin-labeled nascent transcripts; and

isolating said biotin-labeled nascent transcripts by immobilizing same onto streptavidin-labeled iron beads, ~~cleaving said biotin-16-UTP at said cleavable linker to thereby provide a population of nascent RNA transcripts and~~ purifying said RNA transcripts by magnetic separation, cleaving said biotin-16-UTP at said cleavable linker after said magnetic separation to thereby provide a population of nascent RNA transcripts, and quantitatively determining the level of the RNA transcripts by subjecting the RNA transcripts to real-time PCR.

2. (Original) The method of Claim 1 wherein the cells are mammalian cells.

3. (Previously Amended) The method of Claim 1 wherein the RNA transcripts are eluted from the iron beads prior to the quantitative determination.

4. (Cancelled)

5. (Currently Amended) The method of Claim 41 wherein the ~~biotin-labeled~~ RNA transcripts are eluted from the iron beads prior to the quantitative determination.

6. (Previously Added) A method for determining the rate of transcription of one or more transcriptional units in one or more cells, said method comprising:

obtaining from said one or more cells a preparation of nucleic acids comprising said one or more transcriptional units with nascent RNA strands attached thereto,

inhibiting continued transcription of said nucleic acids,

placing said nucleic acids under conditions to permit transcription of said transcriptional unit in the presence of biotin-labeled ribonucleotides, wherein said biotin-labeled ribonucleotides include a cleavable linker between said biotin and said ribonucleotide, to thereby provide a population of biotin-labeled nascent RNA transcripts that include a cleavable linker between said biotin and said ribonucleotide;

isolating said biotin-labeled nascent transcripts by immobilizing said label onto a solid matrix,

cleaving said biotin-labeled ribonucleotide at said cleavable linker to thereby provide a population of nascent RNA transcripts;

and subjecting said nascent RNA transcripts to a real-time polymerase chain reaction to determine the rate of transcription of said one or more transcriptional units.

7. (Previously Added) The method of claim 6, wherein the cleavable linker is a disulfide S-S bridge.

8. (Cancelled)

9. (Cancelled)

10. (Previously Added) The method of claim 6 wherein said preparation of nucleic acids comprises cellular or viral nucleic acids.

11. (Previously Added) The method of claim 6 wherein said nucleic acids are from nuclei, mitochondria, or chloroplasts.

12. (Previously Added) The method of claim 11 wherein said nucleic acids are from nuclei.

13. (Previously Added) The method of claim 6 comprising the additional step of purifying said biotin-labeled nascent RNA transcripts.

14. (Previously Added) The method of claim 6 wherein said inhibiting is accomplished by cooling said nucleic acids.

15. (Previously Added) The method of claim 6 wherein said one or more cells are mammalian cells.

16. (Previously Added) The method of claim 6 wherein said biotin-labeled ribonucleotides are biotin-16-UTP.

17. (Previously Added) The method of claim 6 wherein said biotin-labeled nascent RNA transcripts are eluted from said solid matrix prior to said subjecting step.

18. (Previously Added) A method of detecting a change in activity of one or more transcriptional units, comprising:

determining the rate of transcription in one or more transcriptional units of a first portion of cells according to the method of claim 6,

exposing a second portion of cells to one or more internal or external stimuli,

determining the rate of transcription in one or transcriptional units of said second portion of said cells according to the method of claim 6, and

comparing said rate of transcription of said one or more transcriptional units of said first portion and said one or more transcriptional units of said second portion to detect a change in activity of said one or more transcriptional units.

19. (Currently Amended) A method of claim 18, wherein said one or more internal or external stimuli comprise at least a portion of the nucleic acid sequence of ~~is an~~ endogenous gene.

20. (Currently Amended) A method of claim 18, wherein said one or more internal or external stimuli is an exogenous ~~transgene~~ transgene.

21. (Previously Added) A method of detecting a change in activity of one or more transcriptional units at different stages of cellular development, comprising:
determining the rate of transcription in said one or more transcriptional units according to the method of claim 6 at two or more different stages of cellular development, and
comparing said rates of transcription at said two or more stages of development to detect a change in activity of said one or more transcriptional units.

22. (Currently Amended) A kit for determining the rate of transcription of a transcriptional unit in one or more cells, said kit comprising:
~~enzymes, buffers, and diluents for obtaining nucleic acids;~~
biotin-labeled ribonucleotides, wherein said biotin-labeled ribonucleotides include a cleavable linker between said biotin and said ribonucleotide;
enzymes, buffers, and diluents for transcription of nucleic acids;
a solid matrix;
enzymes, buffers, and diluents for isolating biotin-labeled molecules using said solid matrix; and
enzymes, buffers, and diluents for real time polymerase chain reaction.

23. (Previously Added) A kit for determining the rate of transcription of a transcriptional unit in one or more cells, said kit comprising:
~~enzymes, buffers, and diluents for obtaining nucleic acids;~~
biotin-labeled ribonucleotides;

enzymes, buffers, and diluents for transcription of nucleic acids;

a solid matrix, wherein said matrix includes a cleavable linker;

enzymes, buffers, and diluents for isolating biotin-labeled molecules using said solid matrix, wherein said matrix includes a cleavable linker; and

enzymes, buffers, and diluents for real time polymerase chain reaction.

24. (Previously Added) The kit of claim 22, further comprising enzymes, buffers, and diluents for purifying nucleic acids.

25. (Previously Added) The kit of claim 22, further comprising enzymes, buffers, and diluents for obtaining nuclei, mitochondria, and chloroplasts.

26. (Previously Added) The kit of claim 22, further comprising enzymes, buffers, and diluents for obtaining nuclei.

27. (Previously Added) The kit of claim 22, further comprising instructions for using said kit.

28. (New) The kit of claim 22, further comprising a sucrose buffer.

29. (New) The kit of claim 23, further comprising a sucrose buffer.

30. (New) A method for determining the rate of transcription of one or more transcriptional units in one or more cells, said method comprising:

obtaining from said one or more cells a preparation of nucleic acids comprising said one or more transcriptional units with nascent RNA strands attached thereto,

inhibiting continued transcription of said nucleic acids,

placing said nucleic acids under conditions to permit transcription of said transcriptional unit in the presence of biotin-labeled ribonucleotides to thereby provide a population of biotin-labeled nascent RNA transcripts;

isolating said biotin-labeled nascent transcripts by immobilizing said label onto a solid matrix, wherein said matrix includes a cleavable linker;

cleaving said matrix at said cleavable linker to thereby provide a population of biotin-labeled nascent RNA transcripts;

and subjecting said biotin-labeled nascent RNA transcripts to a real-time polymerase chain reaction to determine the rate of transcription of said one or more transcriptional units.

31. (New) The method of claim 30, wherein the cleavable linker is a disulfide S-S bridge.

32. (New) A kit for determining the rate of transcription of a transcriptional unit in one or more cells, said kit comprising:
biotin-labeled ribonucleotides;

enzymes, buffers, and diluents for transcription of nucleic acids;

a solid matrix, wherein said matrix includes a cleavable linker;

enzymes, buffers, and diluents for isolating biotin-labeled molecules using said solid matrix, wherein said matrix includes a cleavable linker; and

enzymes, buffers, and diluents for real time polymerase chain reaction.